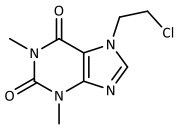
SUPPLEMENTARY INFORMATION

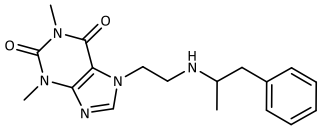
Vaccine-driven pharmacodynamic dissection and mitigation of Captagon psychoactivity

Chemical Analysis. Reactions using microwave energy heating were run on an Explorer 12 Hybrid (CEM). Final chemical compounds synthesized in the course of this study were isolated at >95% purity following HPLC separation (5-95% ACN in H_2O; 60 min; 10 mL/min) using an Agilent 1260 Infinity system and Vydac C18 column (4.6 x 250 mm). Nuclear magnetic resonance (^{1}H NMR (500/400 MHz), ^{13}C NMR (125/100 MHz)) spectra were determined on either a Bruker 500 or 400 instrument. Chemical shifts for ^{1}H NMR are reported in parts per million (ppm) relative to chloroform-*d* (7.26 ppm), Methanol-d_4 (3.31 ppm), or DMSO-*d6* (2.50 ppm) and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts for ^{13}C NMR were reported in ppm relative to the signal at 77.16 ppm for chloroform-*d,* 49.00 for Methanol-d_4, or 39.52 ppm for DMSO-*d6*. Electrospray Ionization (ESI) mass were obtained on a ThermoFinnigan LTQ Ion Trap. Matrix-assisted Laser Desorption/Ionization (MALDI) spectra were obtained on an Applied Biosystems Voyager DE.

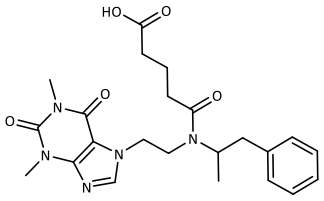
Synthesis of FEN Hapten.



7-(2-chloroethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (**7**). To a microwave vessel containing a solution of **6** (450.4 mg, 2.50 mmol) and 1-chloro-2-iodoethane (952.1 mg, 5.00 mmol) in 1,4-dioxane (5.00 mL), was added potassium carbonate (380.1 mg, 2.75 mmol). The resulting suspension was then stirred and heated to 50 °C using microwave energy for 1 h. The reaction was allowed to cool to room temperature and then quenched with water, treated with 10% sodium thiosulfate to remove excess iodine and extracted into $CHCl_3$. The organic solvent was then dried over Mg_2SO_4, filtered, and excess solvent was removed via rotary evaporation. The resulting material was purified by HPLC to generate the product **7** (410 mg, 1.7 mmol) in 67% yield. $C_9H_{11}ClN_4O_2$; HRMS (ESI) *m/z* calculated = 243.0643; found = 243.0654. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.13 (s, 1H), 4.57 (t, J = 5.7 Hz, 2H), 4.03 (t, J = 5.7 Hz, 2H), 3.43 (s, 3H), 3.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 154.44, 150.93, 148.59, 143.25, 105.77, 47.74, 43.56, 29.46, 27.58.

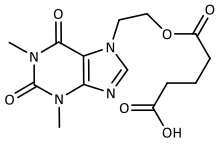


1,3-dimethyl-7-(2-((1-phenylpropan-2-yl)amino)ethyl)-3,7-dihydro-1H-purine-2,6-dione (**1**). To a microwave vessel containing a solution of **7** (350.0 mg, 1.00 mmol), and **2** (135.2 mg, 1.00 mmol) in DMF (5.00 mL) were added potassium iodide (332.0 mg, 2.00 mmol) and potassium carbonate (276.42 mg, 2.00 mmol). The resulting suspension was then stirred and heated to 150 °C using microwave energy for 20 min. The reaction was allowed to cool to room temperature, then quenched with water and extracted into $CHCl_3$. The organic solvent was then dried over Mg_2SO_4, filtered, and excess solvent was removed via rotary evaporation. The resulting material was purified by HPLC to generate the product **1** (292 mg, 0.86 mmol) in 85% yield. $C_{18}H_{23}N_5O_2$; HRMS (ESI) *m/z* calculated = 342.1924; found = 342.1924. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.95 (s, 1H), 7.35 – 7.20 (m, 4H), 7.19 – 7.06 (m, 2H), 4.71 (t, *J* = 5.4 Hz, 2H), 3.82 – 3.58 (m, 3H), 3.58 (s, 3H), 3.38 (s, 3H), 3.11 (dd, *J* = 13.7, 5.8 Hz, 1H), 2.80 (dd, *J* = 13.7, 8.7 Hz, 1H), 1.32 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.03, 151.08, 148.74, 142.78, 134.68, 129.26, 129.19, 127.88, 106.77, 77.16, 57.30, 45.43, 44.27, 39.74, 30.46, 28.60, 16.05.



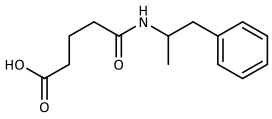
5-((2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)ethyl)(1-phenylpropan-2-yl)amino)-5-oxopentanoic acid (FEN, **8**). To a round bottom flask containing a stirring solution of glutaric anhydride (45.6 mg, 0.40 mmol) in chloroform (4.00 mL), **1** (136.6 mg, 0.40 mmol) was added dropwise. The resulting solution was then stirred for 2 h at 22 °C. The excess solvent was then removed via rotary evaporation. The resulting material was purified by HPLC to generate the product **8** (77 mg, 0.17 mmol) in 42% yield. $C_{23}H_{29}N_5O_5$; HRMS (ESI) *m/z* calculated = 456.2241; found = 456.2240. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.26 – 7.14 (m, 5H), 4.51 – 4.38 (m, 2H), 4.24 (p, *J* = 7.2 Hz, 2H), 3.68 – 3.61 (m, 1H), 3.52 (d, *J* = 3.3 Hz, 3H), 3.38 (s, 3H), 2.79 (d, *J* = 7.3 Hz, 2H), 2.18 – 2.05 (m, 2H), 1.83 – 1.50 (m, 4H), 1.22 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.10, 173.10, 155.38, 149.31, 143.54, 139.43, 129.96, 129.13, 127.18, 107.22, 54.72, 45.66, 33.77, 32.38, 30.32, 28.53, 20.91, 19.73.

Synthesis of THEO Hapten.



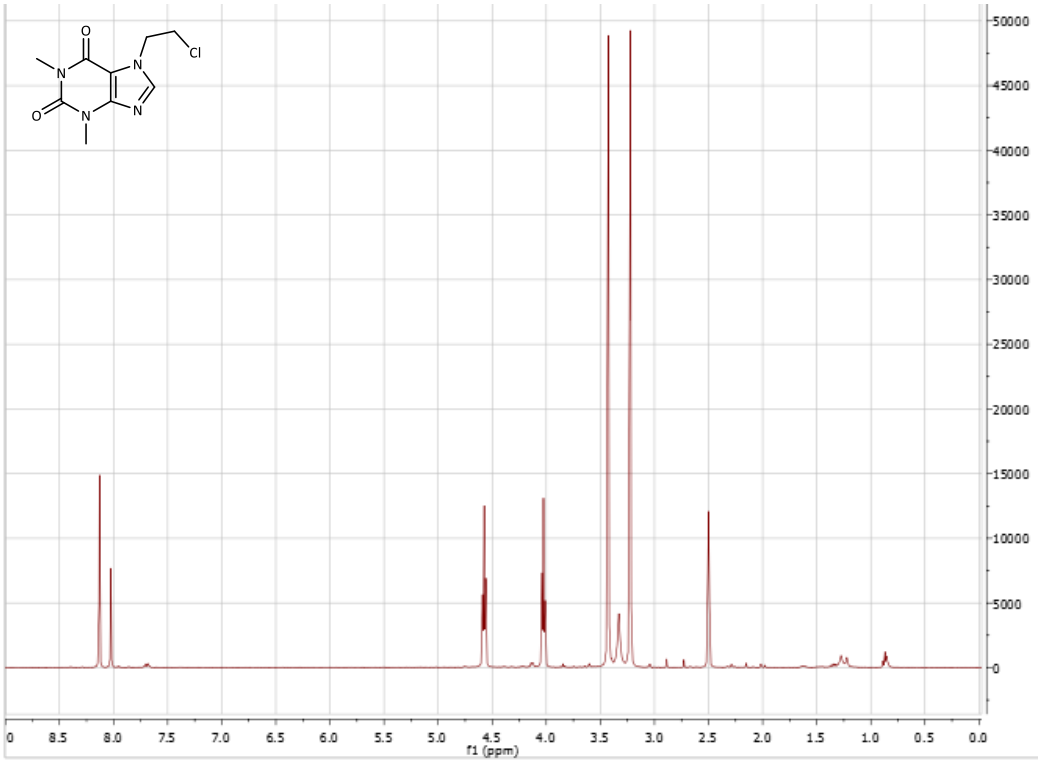
5-(2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)ethoxy)-5-oxopentanoic acid (THEO, **9**). To a microwave vessel containing a solution of **6** (180.2 mg, 1.00 mmol) in DMF (2.00 mL) was added 2-bromoethan-1-ol (500 mg, 4.00 mmol) and sodium hydride (48.0 mg, 2.00 mmol). The resulting solution was then stirred and heated to 150 °C using microwave energy for 1 h. The reaction was allowed to cool to room temperature, quenched with minimal H_2O (40 µL), and the excess solvent was removed via rotary evaporation to generate the crude intermediate 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (193 mg, 0.86 mmol), which was taken directly forward without any additional purification. A portion of this residue (56.1 mg, 0.25 mmol) was suspended in THF (2.00 mL) to which 4-dimethylaminopyridine (15.3 mg, 0.13 mmol) and glutaric anhydride (28.5 mg, 0.25 mmol) were added. The resulting solution was stirred and heated to 90 °C for 16 h. The excess solvent was then removed via rotary evaporation. The resulting material was purified by HPLC to generate the product **9** (28 mg, 0.08 mmol) in 33% yield. $C_{14}H_{18}N_4O_6$; HRMS (ESI) *m/z* calculated = 339.1299; found = 339.1297. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (s, 1H), 4.50 (d, *J* = 5.1 Hz, 2H), 4.39 (d, *J* = 5.1 Hz, 2H), 3.42 (s, 3H), 3.23 (s, 3H), 2.27 (t, *J* = 7.4 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 1.69 – 1.62 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.91, 172.10, 154.47, 150.98, 148.41, 142.95, 106.02, 62.30, 45.38, 32.53, 29.44, 27.56, 19.69.

Synthesis of AMPH Hapten.

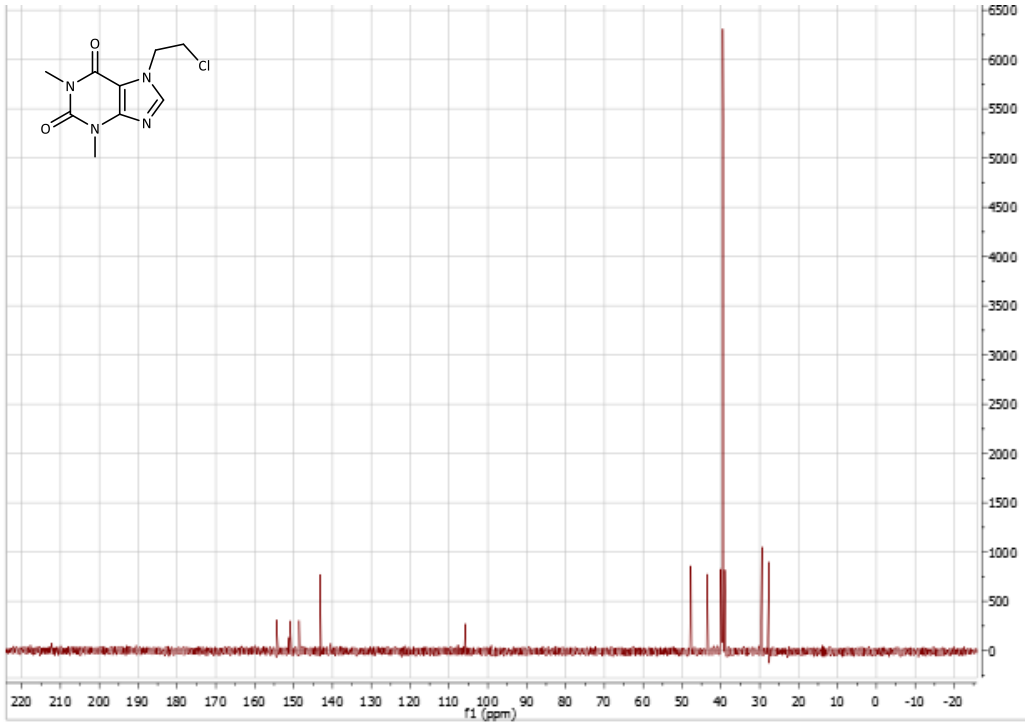


5-oxo-5-((1-phenylpropan-2-yl)amino)pentanoic acid (AMPH,**10**). To a microwave vessel containing a stirring solution of glutaric anhydride (45.6 mg, 0.40 mmol) in THF (4.00 mL), **2** (54.1 mg, 0.40 mmol) was added dropwise. The resulting solution was then stirred and heated to 90 °C using microwave energy for 1 h. The excess solvent was then removed via rotary evaporation. The resulting material was purified by HPLC to generate the product **10** (44 mg, 0.18 mmol) in 44% yield. $C_{14}H_{19}NO_3$; HRMS (ESI) *m/z* calculated = 250.1438; found = 250.1434. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, *J* = 8.1 Hz, 1H), 7.30 – 7.23 (m, 2H), 7.21 – 7.14 (m, 3H), 3.95 (dt, *J* = 14.2, 6.9 Hz, 1H), 2.71 (dd, *J* = 13.2, 7.1 Hz, 1H), 2.59 (dd, *J* = 13.3, 6.8 Hz, 1H), 2.12 (t, *J* = 7.4 Hz, 2H), 2.08 – 1.97 (m, 2H), 1.65 (p, *J* = 7.3 Hz, 2H), 1.00 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.14, 170.63, 139.09, 129.07, 128.04, 125.93, 45.69, 41.87, 34.55, 32.93, 20.68, 20.14.

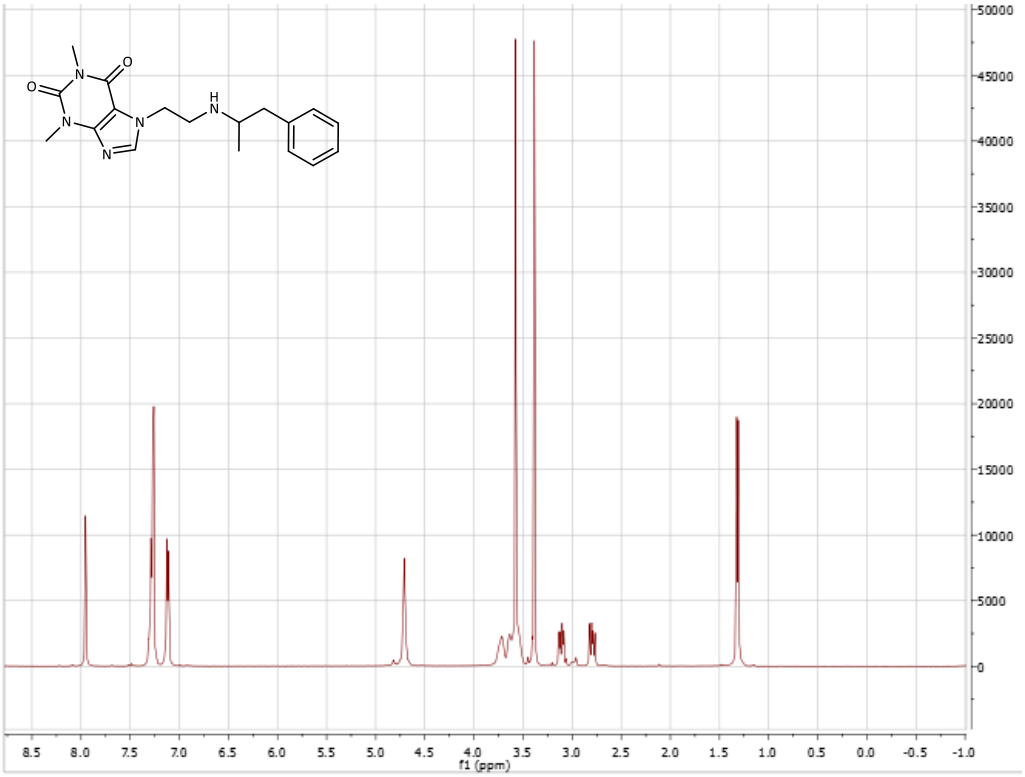
NMR SPECTRA



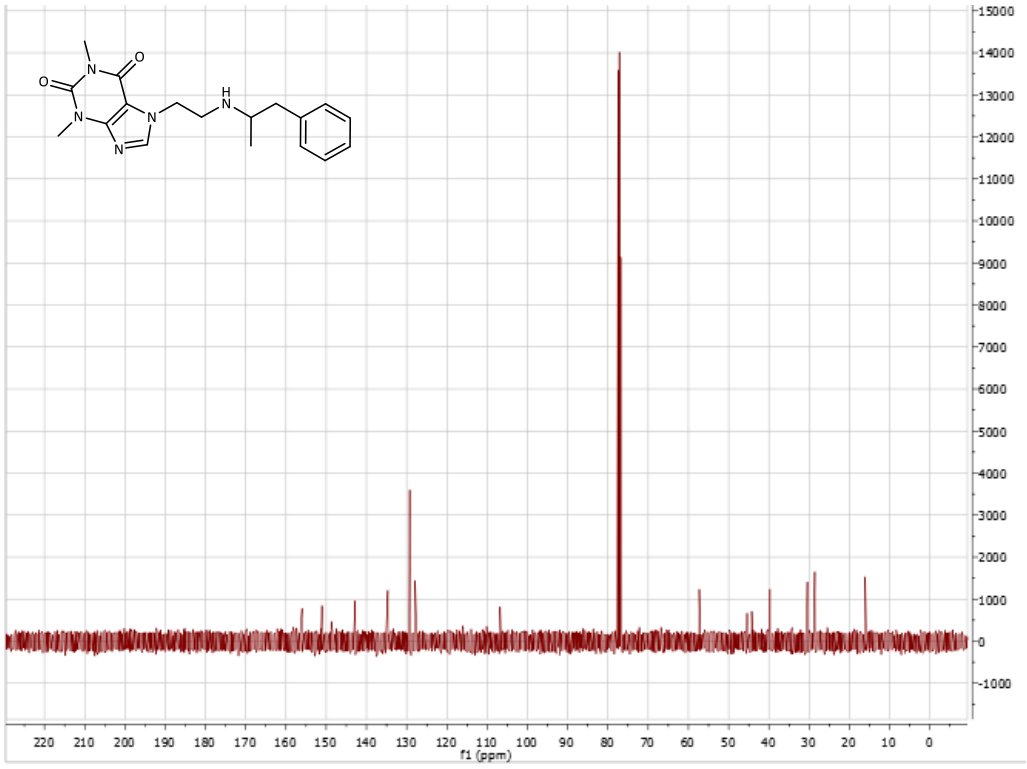
Supplementary Figure 1. ^{1}H NMR for compound **7**



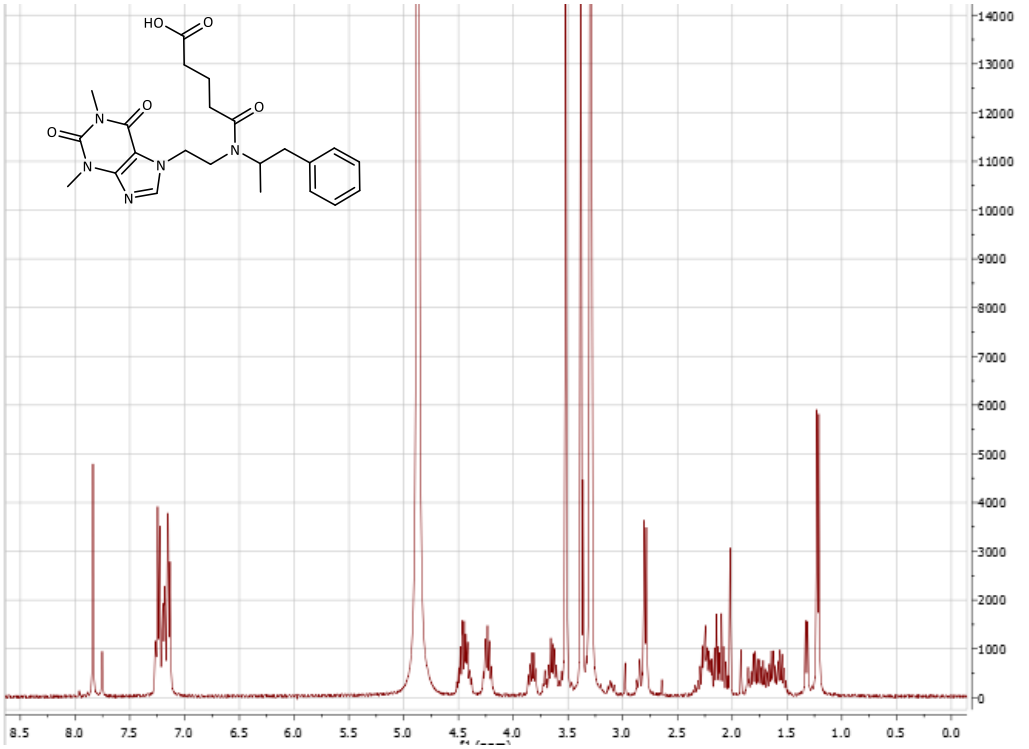
Supplementary Figure 2. ^{13}C NMR for compound **7**



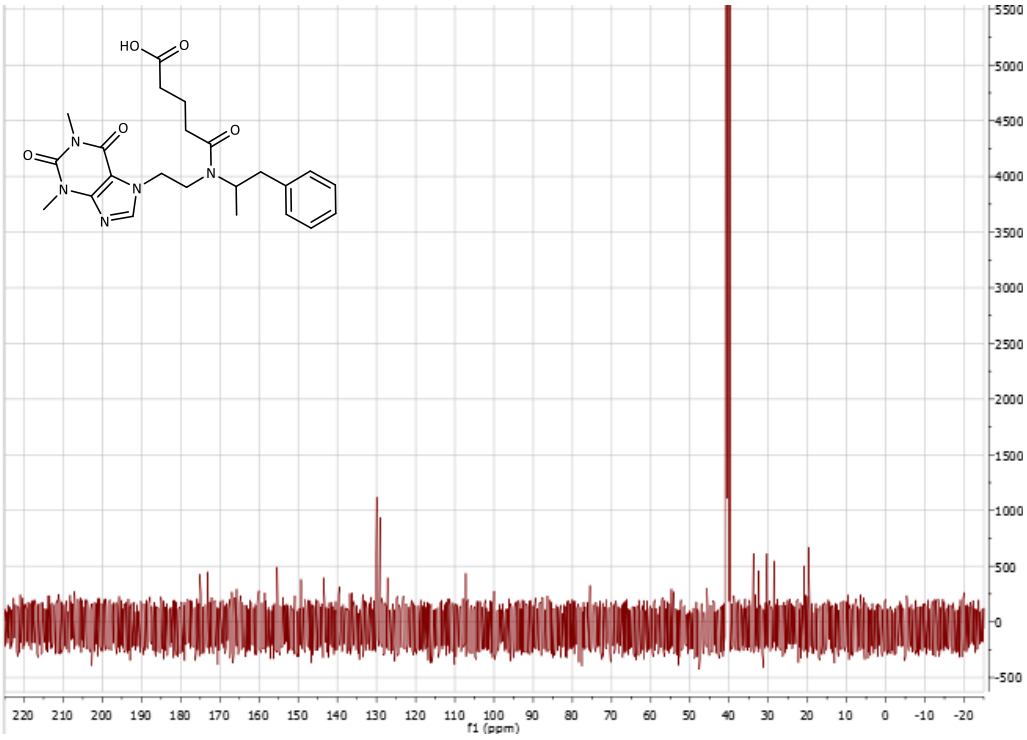
Supplementary Figure 3. ^{1}H NMR of compound **1**



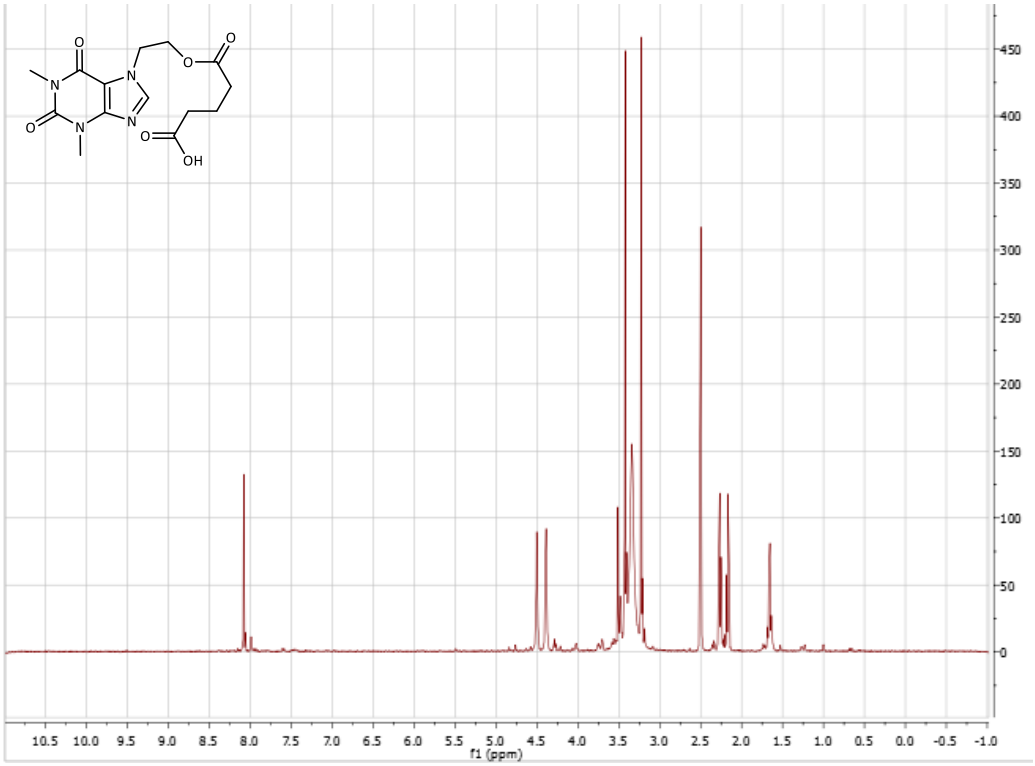
Supplementary Figure 4. ^{13}C NMR of compound **1**



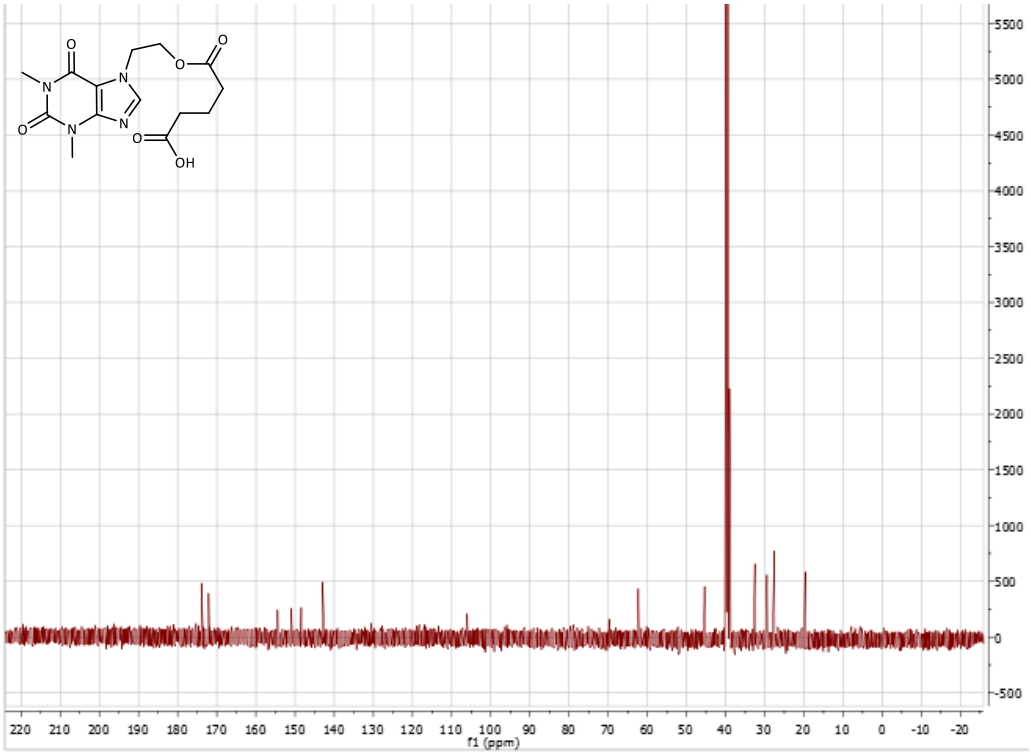
Supplementary Figure 5. ^{1}H NMR of compound **8**



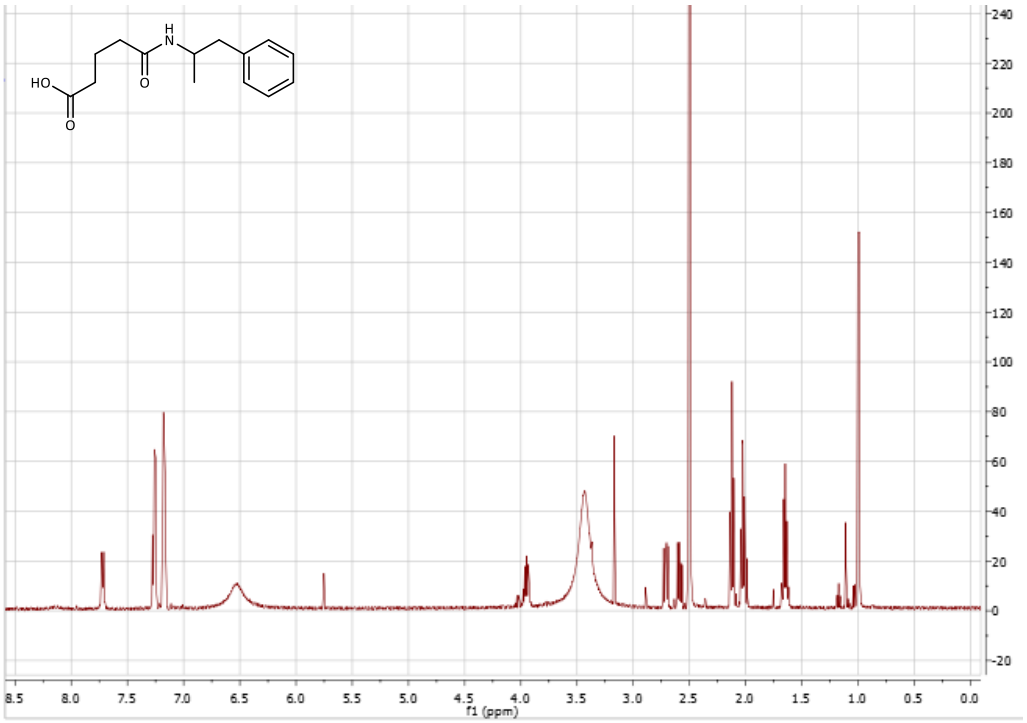
Supplementary Figure 6. ^{13}C NMR of compound **8**



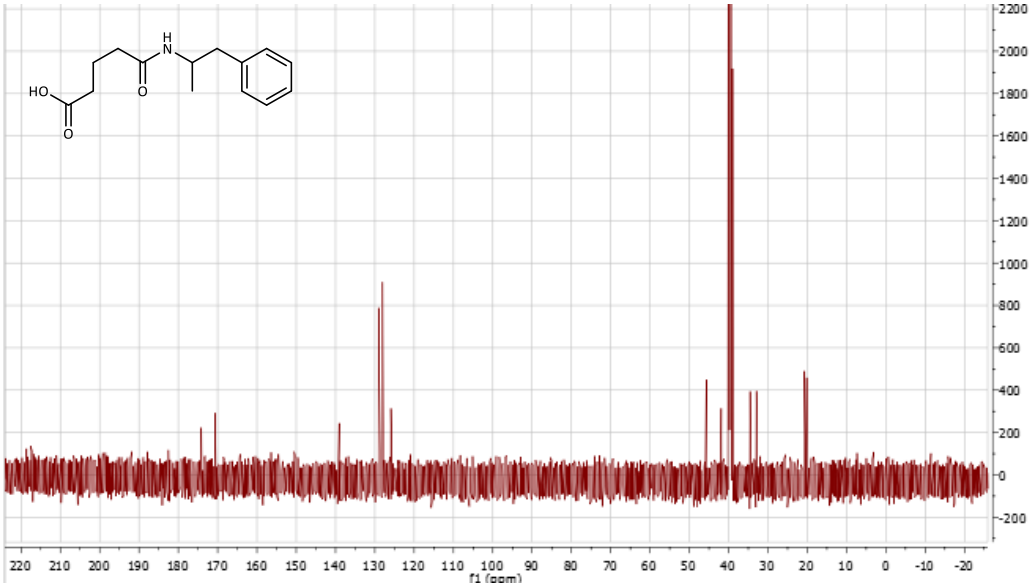
Supplementary Figure 7. ^{1}H NMR of compound **9**



Supplementary Figure 8. ^{13}C NMR of compound **9**



Supplementary Figure 9. ^{1}H NMR of compound **10**



Supplementary Figure 10. ^{13}C NMR of compound **10**